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Kudoa tetraspora n.sp. (Myxosporidea : Protozoa) parasitic in the brain tissue of *Mugil cephalus*

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Abstract. A new species of histozois myxosporidean, *Kudoa tetraspora*, infecting the tissue around the optic lobes of a fish, *Mugil cephalus* is, described. This is the second report of a species of *Kudoa* from India and the second in the world which establishes around the brain. The cysts range varies from 0·3–1·5 mm in diameter and are attached superficially to the tissue around the optic lobes. The spores are quadrate in polar view each measuring 9·0 × 9·0 μm and contains 4 polar capsules. The polar filaments are thick and each measures 10·0–12·0 μm in length. The spores are characteristically arranged in groups of four for which the name 'tetraspora' is proposed.

Keywords. *Kudoa tetraspora* n.sp.; Myxosporidea; brain parasite; *Mugil cephalus.*

1. Introduction

The genus *Kudoa* was established by Meglitsch (1947) for a myxosporidean parasite, *K. clupeidae* (Hahn 1917) found in the body muscles of *Clupea harengus* and *Brevioortia tyrannus*. Since then several species of the genus have been described from different parts of the world. Among them *Kudoa cerebralis* Paperna and Zwerner (1974) is the only species which establishes around the brain and spinal cord and not in the muscle or kidney tissue like the other known species of *Kudoa*. The only previous report of a species of *Kudoa* from India is *K. chilakensis* by Tripathi (1952) from the muscles of the peritoneum of the oesophageal region of *Strongylura strongylura*. The present account deals with the morphology and life-history of a second species of *Kudoa* from India and the second report of a species from the world which establishes around the brain.

2. Materials and methods

The host fish, *Mugil cephalus*, ranging in size from 10–25 cm in length was obtained from the local fish market. A routine examination of the different parts of the body for protozoan parasites showed small cysts ranging in size from 0·3–1·5 mm in diameter attached superficially to the brain tissue near the optic lobes. They were later identified as developmental stages of a myxosporidian parasite belonging

to the genus *Kudoa*. Smears were prepared from cysts of different sizes, fixed in acetone-free methyl alcohol andstained with Giemsa or Loeffler's methylene blue. Smears were also wet-fixed in Schaudinn's or Carnoy's fluid, hydrolysed in 1N HCl at 60° C for 10 min and stained with Heidenhain's iron haematoxylin and Feulgen respectively. Isolated cysts as well as cysts with adjacent tissue were fixed in alcoholic Bouin's fluid, sectioned at 8 μm thickness and stained with haematoxylin eosin. Sections were also stained with PAS-light green and Azan.

Observations on the fresh spores were made by bursting the cysts on a slide and examining them under the pressure of a cover slip. The spores were negatively stained with India ink to detect the presence of any mucuous envelope for the spores.

3. Observations

A total number of 40 *Mugil cephalus* ranging in size from 10–25 cm were collected during April–May 1978 and examined for myxosporidian parasites and 10 of them were infected with a new species of *Kudoa*. Only 2 or 3 cysts were found embedded superficially near the optic lobes in each fish and they range in size from 0·3–1·5 mm in diameter. Sections of the cysts showed that the cyst wall is made up of three layers; an outer layer 3·0–5·0 μm thick consisting of a double layer of collagen enclosing a reticular material of a similar nature and a few cells, a middle layer 10·0–15·0 μm thick containing flattened cells with conspicuous nuclei and nucleoli and an inner layer 8·0–10·0 μm thick which is hyaline and eosinophilic (figures 1 and 13). Sections of cysts having a diameter of less than 0·5 mm showed the developmental stages while the larger cysts showed the spores. We have examined numerous sporonts at different stages of development but have not been able to ascertain the actual number of spores formed from each sporont. Paperna and Zwerner (1974) state that the fully developed sporonts give rise to 2 spores each with 4 polar capsules. We are, however, tempted to believe that each sporont may give rise to either 2 or 4 spores each with 4 polar capsules (figures 8, 11 and 12). In smears of cysts prepared by rupturing them on a slide and allowing the contents to spread on their own (instead of spreading mechanically as is usually done in preparing smears) and in sections of cysts the spores were arranged in groups of 2 or 4, in the latter case they are arranged in the form of a rosette, with the narrower ends directed towards the centre. Such smears when stained with Feulgen-light green showed a delicate membrane surrounding the group of 4 spores. In smears prepared in the conventional manner the groups of spores tend to separate, the spore wall bursts and the polar capsules escape to the outside. In many cases numerous isolated polar capsules, with the polar filaments everted are seen in smears.

3.1. *Spores*

The fully developed spore is quadrate in polar view with deep notches extending about 1/3 the distance inside at the sutural lines and measures 9·0 × 9·0 μm and has 4 polar capsules (figure 7). The spore wall is very delicate and bursts under the pressure of the cover slip. The shell valves are demarcated with faint-sutural lines. The polar capsules are club-shaped and are of the same size

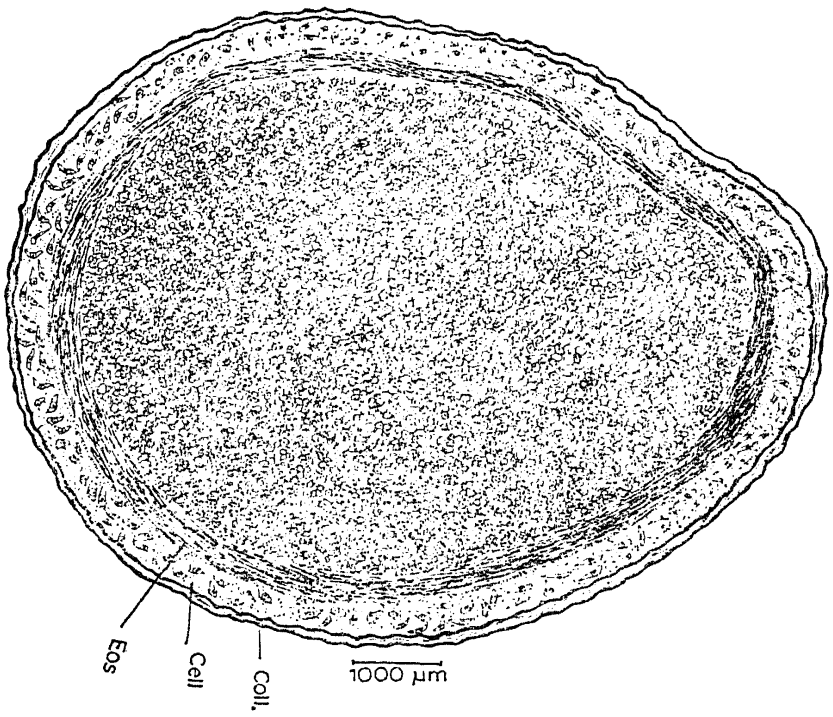
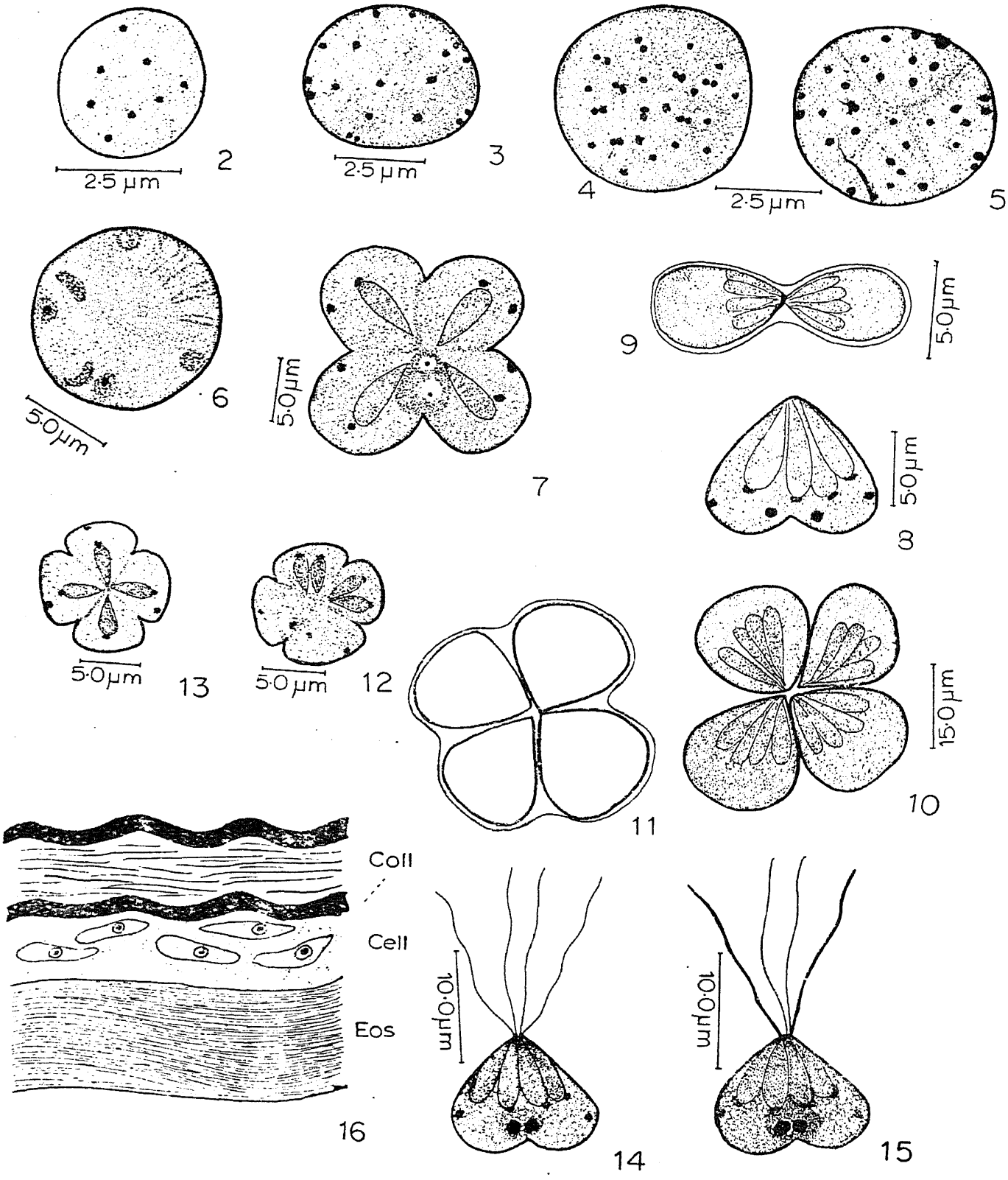


Figure 1. T.S. of a cyst showing numerous spores covered by a wall composed of three layers: Note the outer collagenous, middle cellular and inner eosinophilic layers.

each measuring 3·4–4·0 × 1·5–1·8 µm. The polar filaments are thick and uniformly long each measuring 10·0–12·0 µm (figure 14). About 30% of the spores were examined and found that two of the polar filaments (usually the peripheral ones) are conspicuously thicker than the other two (figure 15). In valvular view the spores appear triangular with rounded corners and bluntly pointed apex. The narrower ends of the polar capsules converge towards the apex of the spore (Fig. 8). The sporoplasm is round or oval and has two conspicuously homogeneous stained nuclei (figures 14 and 15). The spores do not have any mucous or gelatinous envelope as seen in smears negatively stained with India ink.

The earliest sporont observed was oval measuring 5·4 × 6·4 µm and contained 8 deeply stained nuclei (figure 2). The nuclei are surrounded by a very delicate nuclear membrane. Sometimes a clear space is seen around each nucleus. The cytoplasm is finely alveolated and lightly stained and contains a few vacuoles. Sporont showing 16 nuclei and measuring 8·0–8·5 µm in diameter has been observed. Some of the nuclei are adherent to the wall of the sporont and these are probably stages prior to the formation of the pansporoblast which gives rise to 2 sporoblasts and 2 spores eventually (figures 3 and 6). Sporont measuring 12·0–15·0 × 10·0–12·0 µm and containing 32 nuclei has also been observed. The size and structure of the nuclei, the nature and staining properties of the cytoplasm remain the same as in the previous stage (figures 4 and 5). Some of the stages showed the nuclei tending to arrange themselves in four groups (figure 5). The fact that sporonts with 32 nuclei are not uncommon as also the occurrence of spores arranged characteristically in groups of 4, sometimes surrounded by a delicate membrane tempted us to believe that 4 spores are formed from such pansporoblasts. Thus



Figures 2–16. **2.** Sporont with 8 nuclei. **3.** Sporont with 16 nuclei. **4.** Sporont with 32 nuclei. **5.** Sporont with 32 nuclei: Note that the nuclei are arranged in four groups of 8 each. **6.** A pansporoblast prior to the formation of the spores. **7.** A typical spore: polar view. **8.** A typical spore: valvular view. **9.** A group of 2 spores surrounded by a delicate membrane. **10.** A group of 4 spores stained with Loeffler's methylene blue. **11.** A group of 4 spores stained with Feulgen–light green: Note the thin membrane surrounding the spores. **12.** A spore showing the polar capsules evenly distributed. **13.** A spore showing the polar capsules distributed one each in each of the quadrants. **14.** A valvular view of the spore-polar filaments released. **15.** A valvular view of the spore: Note the peripheral filaments are thicker. **16.** A portion of the cyst wall—note the three layers (not to scale). (Cell : Cellular layer ; Coll : Collagen layer ; Eos : Eosinophilic layer).

t would appear that the pansporoblast in the present form may give rise to either or 4 spores (figures 9, 10 and 11). The distribution of the polar capusles within he spore also shows some variation. Usually there is one polar capsule in each uadrant of the spore (figure 13) but sometimes two polar capsules may be found n two of the quadrants of the spore leaving the other two quadrants empty figure 12).

. Discussion

Meglitsch (1947) established the genus *Kudoa* for members belonging to the family Chloromyxidae which are histozoic and whose spores are quadrate or stellate in polar view. He designated *K. clupeidae* (Hahn 1917) as the type species and transerred 8 species of *Chloromyxum* to the new genus created by him. The only pecies of *Kudoa* described from India is *K. chilakensis* Tripathi, 1952 from the nuscles of the peritoneum of the oesophageal region of *Strongylura strongylura.* The size of the spores, the site of infection and the host fish are all different when ompared to the present form. Rigdon and Hendricks (1955) reported *Kudoa* p. from the muscles of a single specimen of *Mugil cephalus* but they have not iven the spore measurements and hence a comparison with the present form is ot possible. Although both are from similar hosts the site of infection is different. Paperna and Zwerner (1974) reported *K. cerebralis* from the tissue, the brain and pinal cord of *Morone saxatilis* (Walbaum). The site of infection in the present orm and *K. cerebralis* is same but the hosts belong to different genera and species nd are from different geographical localities. Further the spores in *K. cerebralis* re smaller than in the present form. For these reasons the present form is escribed as a new species and the name *Kudoa tetraspora* n. sp. is proposed.

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Hahn C W 1917 On the sporozoan parasites of the fishes of Woods Hole and vicinity. III. On the *Chloromyxum clupeidae* of *Clupea harengus* (Young), *Pomolobus pseudoharengus* (Young) and *P. aestivalis* (Young); *J. Parasitol.* **4** 13–20

Meglitsch P A 1947 Studies on Myxosporidea from the Beaufort region. II. Observations on *Kudoa clupeidae* (Hahn) gen. Nov.; *J. Parasitol.* **33** 265–277

Paperna I and Zwerner D E 1974 *Kudoa cerebralis* sp. n. (Myxosporidea, Chloromyxidae from the striped Bass, *Morone saxatilis* (Walbaum); *J. Protozool* **21** 15–19

Rigdon R H and Hendricks J W 1955 Myxosporidea in fish in waters emptying into Gulf of Mexico; *J. Parasitol.* **41** 511–518

Tripathi Y R 1952 Studies on the parasites of Indian fishes: 1. Protozoa: Myxosporidea together with a check list of parasitic protozoa from Indian fishes; *Rec. Indian Mus.* **50** 63–68